

The University of Chicago

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WOMEN ON DIETS CONTAINING
VARYING LEVELS OF THE
B VITAMINS

A PART OF A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS
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RIBOFLAVIN EXCRETIONS OF YOUNG WOMEN ON DIETS CONTAINING VARYING LEVELS OF THE B VITAMINS¹

MARGARET V. DAVIS²

Department of Home Economics, University of Chicago, Illinois

ONE FIGURE

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Although a number of studies have been made on the excretion of riboflavin by persons maintained on a controlled intake of the vitamin, only a few have had women as subjects. Sebrell, Butler, Wooley and Isbell ('41) maintained a group of ten women on a diet containing 0.21 mg riboflavin per 1000 Cal. for a period of 4 to 8 months. The daily excretion averaged 74 μ g riboflavin per day. Six of the women developed cheilosis. On the basis of added amounts of the vitamin required to prevent or cure cheilosis and of the urinary excretion on different levels of intake, these workers suggested that the riboflavin requirement was about 3 mg/day, or about 1.2 to 1.5 mg/1000 Cal. The results of a brief experiment on four college girls by Strong, Feeney, Moore and Parsons ('41) appeared to confirm this recommendation. On a dietary intake of 1 to 2 mg riboflavin the daily urinary excretion was 50 to 150 μ g, in comparison to a 500 to 800 μ g daily excretion observed in a group of students and faculty members eating

¹ The expenses of this study were defrayed by funds granted by the National Dairy Council on behalf of the American Dairy Association and by the Williams-Waterman Fund of the Research Corporation.

² The data reported in this paper were taken from a thesis presented by Margaret V. Davis to the Faculty of the Division of Biological Sciences of the University of Chicago in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

their normal diet. More recent work indicates that these early recommendations were high, however. The daily urinary excretion of riboflavin and the response to a test dose of the vitamin by groups of women maintained on different levels of riboflavin intake were studied by Williams, Mason, Cusick and Wilder ('43). Urinary excretion fell to about 100 μ g/day and test dose returns were markedly lowered on an intake of 0.35 mg/1000 Cal. On an intake of 0.5 mg/1000 Cal., daily excretion fell to low levels but test dose returns were not affected as greatly as on the smaller amount. On an intake of 0.8 mg/1000 Cal. there was no noticeable decrease in the excretions. These workers suggested that the requirement of riboflavin by women was not less than 0.5 mg, nor more than 0.8 mg/1000 Cal., or a total of 1.0 to 1.6 mg per day.

In the present work the riboflavin excretion of a group of healthy young women, living on a diet in which the amount of riboflavin was progressively increased, was followed over a period of 8 months. The young women were university students, leading a life characteristic of such a group. Efforts were made to insure that the experimental procedures should result in as little change as possible in their usual habits. It was hoped that such a study would yield further information on the utilization of and requirement for riboflavin by normal young women, insofar as such requirements can be judged on the basis of excretion of the vitamin on known levels of intake. That the excretion level of a vitamin reflects the tissue concentration of that vitamin has been demonstrated in the case of thiamine by Hulse and co-workers ('44).

METHODS OF STUDY

Altogether twelve young women between the ages of 19 and 32 years served as subjects during the course of the study. Of the nine original subjects, three were followed throughout the entire 8-month period, the others for periods of 3½ to 5 months. Three months before the end of the study it was decided that at the level of riboflavin intake planned for the remainder of the time, new subjects might be included after

a short period of adjustment to the diet. Three young women were selected to serve as subjects at this time, together with three who had been on the diet since the beginning.

Each subject received a complete physical and neurological examination some time during the first 2 weeks of the study.³ This examination was repeated after a period of 7 weeks on the experimental diet. At the end of 3½ months a special examination was made for any signs of riboflavin deficiency.

The results of the preliminary examinations showed the young women to be in a good general state of health. Two persons (subjects 7 and 10) were found to have hemoglobin values somewhat below normal, and three deviated considerably from the average weight for their height. Subject 2 was 17% overweight, while subjects 1 and 3 were approximately 20% underweight. There were no other negative findings.

The experiment was divided into five periods. During the first period, which lasted for 106 days, the average intake of riboflavin was 0.29 mg/1000 Cal. After the first 59 days on this level certain changes were made in the diet which increased the intake of thiamine, but did not change that of riboflavin. The times before and after this change have been designated as periods I-a and I-b. In period II, 45 days in length, the riboflavin intake was increased to an average of 0.49 mg/1000 Cal., and in period III, which lasted 41 days, to an average of 0.66 mg/1000 Cal. In period V, 14 days long, the diet was supplemented with 6 mg riboflavin, 4 mg thiamine, 0.5 mg pantothenic acid, and 10 mg niacin per day. This period of "supercharging" was followed by a return, for 20 days, to approximately the same level of intake as in period III.

Except during period IV, when the supplements of synthetic vitamins were given, and during periods III and V when a daily supplement of approximately 100 µg of thiamine was given, all variations in the vitamin content of the diet were made through changes in the kinds and amounts of foods.

³ Grateful acknowledgment is made to Dr. Oscar Kreisler, who kindly made these examinations.

This was done because it was considered that the results would be more applicable to practical situations than if the synthetic vitamin were used.

The menus for the experimental diet were worked out on the basis of 3-day periods. The same foods were served each 3 days, although their method of preparation was varied.

TABLE 1

Amounts of basic foods served every 3 days during each experimental period.

FOOD	PERIOD				FOOD	PERIOD			
	I-a	I-b	II	III and V		I-a	I-b	II	III and V ¹
	gm	gm	gm	gm		gm	gm	gm	gm
Beef	150	150	200	200	Rhubarb	...	...	100	...
Chicken	75	75	100	100	Berries	...	...	...	100
Carrots	70	70	100	100	Grapefruit				
Beets	70	70	...	...	juice	120	120	...	...
Green beans	60	60	100	100	Tomato juice	120	...	120	120
Peas	...	...	100	100	Apple juice	120	...	...	...
Potatoes	100	100	300	300	Orange juice	...	200	360	360
Head lettuce	100	100	190	210	Rice	200	200	...	...
Celery	30	30	20	...	Farina	340	...	...	...
Pineapple	72	72	200	200	Oatmeal	...	300	400	400
Apple	200	200	100	...	Cream	<132	<110	<110	<110
Peaches	...	...	100	100	Milk	...	...	600	1050 ²
Pears	...	...	...	100	Jam	<150	<150	<150	<150

¹ The same general dietary pattern was followed during period IV, when supplements were given, as during periods III and V. Some variation was allowed in period IV, however.

² In an attempt to keep vitamin/calorie ratios similar for all subjects, subject 2 received 600 gm of milk every 3 days, and subject 12, 900 gm every 3 days during periods III and V.

Foods which contained considerable amounts of riboflavin, such as meat, milk, fruits and vegetables, were served in equal quantities to each person. The amounts of these foods served in each 3-day period will be found in table 1.

In order to increase the caloric intake, cake and cookies and usually some form of pastry were served in each 3-day period in approximately equal amounts to each person. Bread and

butter were allowed ad libitum as required to meet individual caloric needs. All of the baked products were prepared in the laboratory using unenriched flour, low vitamin yeast, water, and minimal amounts of egg.

A complete record of food consumption was kept for each individual. No food was eaten except that served at meals, although outside consumption of certain beverages was permitted. A careful record of these was kept by each subject, and they were included when the caloric value of the diet was calculated.

The basal diet was planned to be low in both riboflavin and thiamine. The niacin content of the diet in the early periods was only slightly below the present recommended allowance of 12 mg per day for moderately active women. It was assumed that the diet was adequate in other B-vitamins for which requirements are not known. Supplements of di-calcium phosphate, ferric pyrophosphate, vitamin A and ascorbic acid were given. Complete details of the nutritive value of the diet and of its supplementation will be found in table 2. The amount of iron given in period I-b was increased over that in period I-a in connection with a study on serum iron which was made on the subjects during period I.

Preliminary observations on the average daily excretion of riboflavin, the fasting 1-hour excretion, and the return of a test dose were made during the week prior to the start of the experimental diet. The subjects continued on a self-chosen diet during this week, except that they were asked not to eat liver or pork during the period.

During the course of the experiment 72-hour urine collections were made each week, except during the first week of a new experimental period. The urine samples were collected in brown glass bottles, and preserved with acetic acid, toluene, and a few drops of chloroform. One-fifth of the entire 3-day sample was held under refrigeration until analyzed.

During the last week in each experimental period two 1-hour specimens of urine voided before breakfast were collected. On one morning during this week a low-vitamin breakfast

TABLE 2

Length of periods, number of subjects, and composition of the diet throughout the study.

	PERIOD					
	I-a	I-b	II	III	IV	V
Length (days)	59	47	45	41	14	20
Number of subjects	9	9	5	6	6	6
<i>Average intake</i> ¹						
Riboflavin — mg/1000 Cal.	0.29	0.28	0.49	0.66	4.10 ⁴	0.63
Thiamine — mg/1000 Cal.	0.14	0.20	0.36	0.51 ³	2.80 ⁴	0.54 ³
Niacin — mg	11.0	9.7	10.6	14.3	F ⁵ 14.3 S 10.0	14.3
Pantothenic acid — mg	2.3	2.4	3.5	4.3	F 4.3 S 0.5	4.7
Protein — gm	49	51	61	62	62	62
Fat — gm	73	78	82	68	68	68
Carbohydrate — gm	311	307	289	247	247	247
Vitamin A — I.U. ²	F 4200 S	F 4200 S	F 6300 S 5000	F 6500 S 5000	F 6500 S 5000	F 6500 S 5000
Ca — gm	F 0.17 S 0.35	F 0.22 S 0.24	F 0.47 S 0.24	F 0.67 S 0.24	F 0.67 S 0.24	F 0.67 S 0.24
Fe — mg	F 6.9 S 4.0	F 7.6 S 8.0	F 10.8 S ..	F 11.3 S ..	F 11.3 S ..	F 11.3 S ..
Vitamin C — mg	F42.4 S50.0	F70.8 S50.0	F113.4 S 25.0	F115.3 S 25.0	F115.3 S 25.0	F115.3 S 25.0

¹ Figures for riboflavin, thiamine, nicotinic acid and pantothenic acid determined by analysis; those for other nutrients by calculation.

² International Units.

³ Figure includes supplement of approximately 100 µg thiamine per day.

⁴ Figure includes supplements — 6 mg riboflavin, 4 mg thiamine.

⁵ F — amount supplied by food; S — amount supplied by supplement.

was given, and the urine excreted during the following 4 hours was collected. This constituted a "4-hour control sample." On the next to last morning of each period a test dose of riboflavin was given orally with the same low-vitamin breakfast and a 4-hour sample collected.⁴ The size of the test dose was 0.02 mg/kg body weight, which gave a total of approximately 1 mg. In calculating the percentage of the test dose which was returned, the riboflavin value of the 4-hour control sample was subtracted from that of the 4-hour sample taken following the test dose.

During the times when urine collections were being made, samples of all foods were saved for analysis. Composites representing one-fifth of the total amount of food taken by each person, with the exception of cereal foods, sugar, and butter, were made at the end of the 3-day periods. The samples were homogenized, brought to approximately pH 1 by the addition of 8 ml concentrated hydrochloric acid per liter of suspension, and autoclaved at 15 pounds pressure for 15 minutes. Cereal foods were analyzed separately and the amount of riboflavin taken by each person in such foods was determined by calculation. This procedure was followed in order that the cereals would not exert any untoward effects on the riboflavin analyses.

Fecal collections, marked by carmine, representing the same period as the last 3-day urine collection in each period except period IV were made by some of the subjects. After the wet weight had been recorded, the feces were placed in a large evaporating dish and covered with 50% alcohol. Succeeding samples were added to the dish, which was kept refrigerated until the collection was complete. The samples were dried to constant weight at 45°C. After drying they were ground and stored in the refrigerator until analyzed.

The riboflavin content of the food composites, urine, and feces was determined by the microbiological method (Snell and Strong, '39; Strong and Carpenter, '42). A modified

⁴Riboflavin was generously supplied by Merck and Company.

basal medium, the same as that used by Oldham, Johnston, Kleiger and Hedderich ('44) for cereal analyses, was used for all analyses in this study.

RESULTS AND DISCUSSION

A summary of the results of the study is found in table 3. Included in this table are the results of (1) the preliminary measurements of urinary excretion of riboflavin, (2) the average daily intake and urinary excretion of the vitamin by each subject during each period, and (3) the average daily fecal excretion, fasting 1-hour excretion and return of a test dose at the end of each period.

Results of preliminary observations. As would be expected among a group of people of different dietary habits, there was considerable variation in the daily urinary excretion of riboflavin during the preliminary period. There appeared to be a relationship between the daily excretion and the amounts of riboflavin-containing foods, particularly of milk, taken by the young women. Subjects 7 and 11, who had the lowest daily excretions, drank little milk. All of the others were accustomed to drinking milk in varying amounts.

Only four of the subjects returned as much as 20% of the test dose, the amount which was suggested by Oldham and co-workers ('44) as indicative, in the case of children, of a satisfactory nutritional status with regard to riboflavin. One of these four returned approximately 35%, which is the amount suggested by Feder, Lewis and Alden ('44) as a normal return of a test dose. It should be noted, however, that in the data presented by Feder et al., the total 4-hour excretion following the administration of the test dose is used in calculating the percentage returned. In the method of calculation used in this paper, where the normal 4-hour excretion is subtracted from the amount excreted in the 4 hours following the test dose, a smaller percentage return would be expected. Although the return of a test dose has been used as a criterion in nearly every study of riboflavin requirements, the size of the test dose, the method of its administration, and

of calculating the return have varied. Because of these variations it is difficult to assess the original nutritional status of the subjects on the basis of test dose returns.

Twenty-four-hour excretions

The 24-hour excretions of riboflavin adjusted to changes in intake quite rapidly. After about 10 days on a new dietary level, the daily excretions reached approximately the level at which they remained throughout the period. One exception was observed during period I-a. This was subject 2, whose excretion of riboflavin during the preliminary period was much higher than that of anyone else. Her riboflavin excretion dropped gradually during the first 6 weeks on the experimental diet and by the seventh week had reached a level at which it continued until the end of period I-b. In the case of subjects 7 and 11, who had shown low daily excretions of riboflavin during the preliminary period, the 24-hour excretion level did not change significantly when the experimental diet was started.

In spite of the relatively large increase in riboflavin intake in period II, from 0.29 to 0.49 mg/1000 Cal., there was only a small increase in the daily excretion of the vitamin. However, when the riboflavin intake was increased in period III to 0.66 mg/1000 Cal., there was a definite rise in excretion in all subjects. As would be expected, an immediate, striking rise in daily excretion occurred in period IV when the diet was supplemented with 6 mg riboflavin. Urine collections were made during the first 3 days and last 3 days of this period. An average of 55% of the total intake of riboflavin was excreted during the first 3 days, and of 66% during the last 3 days. Apparently there was a tendency for the body to "hold on" to some of the extra vitamin at first. Subject 2 excreted a greater proportion of the supplement than did the others; this indicates, perhaps, that her stores were more nearly full than were those of the other subjects.

Following the vitamin supplementation, and at the end of 2 weeks on an average daily intake of 0.63 mg 1000 Cal., the

TABLE 3 — (continued)

SUBJECT	1	2	3	4	5	6	7	8	9	10	11	12	AVERAGE
HEIGHT, CM	165	169	161	170	160	152	164	175	165	158	174	170	
WEIGHT, KG	46.3	71.7	45.8	56.2	56.2	50.8	58.5	62.6	59.0	59.4	63.5	69.4	

Period III

<i>Intake</i>													
Total	1360	1134	1244	...	...	1347	...	...	...	1253	...	1063	1234
Mg/1000 Cal.	0.65	0.64	0.65	...	...	0.63	...	...	...	0.68	...	0.72	0.66
<i>Excretion</i>													
Urinary													
μg/24 hr.	345	214	234	...	...	287	...	...	...	229	...	269	263
Fecal													
μg/24 hr.	415	656	570	...	...	657	...	...	...	..	...	574	574
Fasting													
μg/hour	8	11	15	...	...	14	...	...	...	8	...	9	11
Test dose													
% return	26.5 ²	17.9	12.9	...	...	13.9	...	...	...	11.1	...	15.5	14.3

Period IV

<i>Intake, total</i>													
Week 1	7252	7071	7210	...	...	7213	...	...	...	7169	...	7066	7164
Week 2	7257	7045	7232	...	...	7254	...	...	...	7210	...	7065	7177
<i>Excretion, urinary/24 hr.</i>													
Week 1	4275	4835	3968	...	...	4254	...	...	...	3361	...	2917	3935
Week 2	4847	5805	4958	...	...	4538	...	...	...	4547	...	3823	4753

Period V

<i>Intake</i>													
Total	1291	1086	1254	...	...	1275	...	...	...	1267	...	1065	1206
Mg/1000 Cal.	0.59	0.54	0.68	...	...	0.59	...	...	...	0.59	...	0.76	0.63
<i>Excretion</i>													
Urinary													
μg/24 hr.	403	504	299	..	...	243	...	...	...	212	...	286	325
Fecal													
μg/24 hr.	361	856	611	...	...	628	..	...	...	...	...	540	599
Fasting													
μg/hour	16	15	14	...	...	10	..	...	..	16	...	11	13
Test dose													
% return	18.1	17.0	10.2	...	...	24.6	..	...	...	14.6	...	9.1	15.6

¹Daily intakes and urinary excretions during periods I-a, I-b, II, and III represent the average of 3-day collections made weekly throughout these periods, with the exception of those for subject 3 in periods I-a and I-b which represent a single 3-day collection at the end of these periods.

²This figure is not included in the average. Due to an error the 4-hour collection was not made following the test dose, and a second test dose was given the following day. This is the return of the second test dose.

daily urinary excretion was almost exactly the same as it had been during period III for five of the six subjects. Here again subject 2 was the exception, her daily excretion being almost double that observed in period III. It should be pointed out that the results shown for period V represent only one 3-day collection. Throughout the course of the experiment there were a few cases in which the average daily excretion for a particular week did not fall exactly in line with the results secured during the rest of the period. It is possible that something of this nature may have occurred in the case of this particular collection. In view of the other results secured on subject 2, however, it seems probable that she did have more riboflavin "available" for excretion than did the other subjects.

Fasting 1-hour excretion

During periods I-a and I-b, when the average intake was 0.29 mg/1000 Cal., the fasting 1-hour excretion dropped sharply, and at the end of period I-b it averaged 6 μ g. When the riboflavin intake was increased to an average of 0.49 mg/1000 Cal., the fasting excretion rose to an average of 11 μ g per hour. There was no further increase in the fasting excretion when the intake was increased to 0.66 mg/1000 Cal. in period II, nor was the excretion in period V (13 μ g per hour) following the supplementation in period IV, appreciably higher than in period III.

The fasting 1-hour excretions were also calculated on a unit volume basis as suggested by Feder, Lewis and Alden ('44). It was found that the values for excretion per hour were more constant than the values for excretion per unit volume.

Test dose returns

A sharp drop in test dose returns was found to have occurred at the end of 59 days on an average intake of 0.29 mg/1000 Cal. At that time four of the nine subjects excreted 1% or less of the test dose, while four others returned between 2 and 4%. One person, subject 2 again, showed a much higher

return than anyone else in the group — 11.6%. At the end of period I-b the average return of a test dose was slightly higher, despite the fact that there had been no increase in the amount of riboflavin in the diet. If subject 1, who showed an unusually large increase in test dose return (from 0.3 to 7.0%) is not included, the average returns are 3.1 and 4.2% for periods I-a and I-b, respectively, which still indicate a slight increase. Although the level of riboflavin in the diet was not changed at the end of period I-a, the thiamine was increased from an average of 0.14 to 0.20 mg/1000 Cal. It is possible that this increase in thiamine may have had some effect on the utilization of riboflavin.

At the end of period II there was a sharp increase in the amount of the test dose returned, the average at this time being 11.8%. There was a further increase, to an average of 14.3%, at the end of period III. Apparently no additional benefit was derived from the 2-week period of vitamin supplementation; the average amount returned at the end of period V was 15.6%, practically the same as the average return at the end of period III. Two of the six subjects showed an increased return; the other four returned approximately the same amount or slightly less than in period III. According to the theory of Melnick ('42) this would indicate that the tissue stores of riboflavin had been satisfactorily filled on the 0.66 mg/1000 Cal. level of intake, since the 2-week period of high intake did not affect the test dose return.

Fecal excretion

Although there was considerable variation in the total amount of riboflavin excreted in the feces by different individuals, the level for each person remained relatively constant. Apparently the fecal excretion was not influenced either by changes in the dietary level of riboflavin, or by the fact that the starch content of the diet was higher during the early periods of the study, when larger amounts of cereal foods were necessary to maintain the caloric intake.

That intestinal synthesis of riboflavin did occur is shown by the fact that in some cases the fecal excretion alone was higher than the dietary intake of the vitamin. In a number of other cases the total urinary and fecal excretion was higher than the intake. Whether any absorption or utilization of this synthesized vitamin took place is difficult to determine.

General health

No definite signs or symptoms of riboflavin deficiency were observed at the time of the original examination, or in the examination at the end of period I-a. No cheilosis was observed among any of the subjects at any time, nor did any seborrheic dermatitis occur. The eyes were not examined with a slit lamp, but a magnifying glass failed to reveal any abnormal vascularization of the cornea. During the first examination the tongue of one of the young women (subject 3) was observed to show slight enlargement and reddening of the fungiform papillae, and some indentation of the margin. This condition did not change throughout the study. When the special examination of the mouth, tongue, and eyes was made at the end of period I-b, two other subjects were observed to have a similar condition of the papillae, and one showed slight indentations of the margin of the tongue. Two subjects were found to have slight photophobia when the eyes were exposed to a strong light. One of these was subject 11, whose daily excretion and return of a test dose were low. The other was subject 2, whose excretions were high. All of the changes were slight, however, and the clinician who made the examination stated that they were "not convincing and of doubtful significance."

Constipation was observed by certain of the subjects during periods I-a and I-b, and was severe in several cases. This occurred in spite of the fact that fruit juice, raw or cooked fruit, and a cooked and a raw vegetable were included in the meals each day. The condition was relieved immediately when the diet was changed in period II, at which time both the riboflavin and thiamine contents of the diet were increased.

At the end of period I-b four young women withdrew from the study because of unfavorable subjective symptoms. All had severe constipation and all complained of fatigue, lack of concentration, desire to sleep, and general malaise. It seems likely, however, that these symptoms, if caused by the diet, were due to a low intake of thiamine rather than of riboflavin.

Interpretation of results

A graphic comparison of the average intake and excretion of riboflavin by all of the subjects throughout the course of the study is found in figure 1. From the pronounced drop in

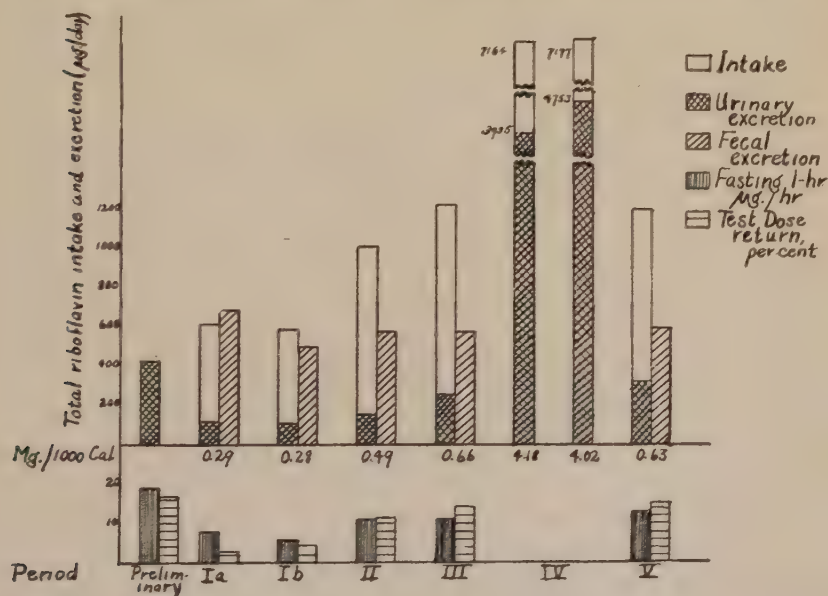


Fig. 1 Average intake and excretion of riboflavin during each experimental period.

daily excretion, fasting excretion, and test dose returns which occurred during period I, when the riboflavin content of the diet was restricted to 0.29 mg/1000 Cal., it seems evident that the riboflavin in the body tissues reached a low level during this period.

Although the 24-hour excretion did not increase greatly when the riboflavin intake was raised to an average of 0.49 mg/1000 Cal., there was a striking increase in both the fasting excretion and the amount of a test dose returned. The data on the test dose returns indicate that the concentration of tissue riboflavin had been increased during this period.

The rise in 24-hour excretion in period III (on an average intake of 0.66 mg/1000 Cal.) was greater in proportion than might have been expected from the dietary increase in riboflavin. The average increase in riboflavin intake in each period, together with the resulting increase in excretion are shown in table 4. The percentage excreted, both of the total intake and of the added dietary riboflavin in each period is also shown in table 4.

TABLE 4

Percentage of the total intake and of the increase in intake which was excreted at each dietary level.

PERIOD	TOTAL INTAKE	INCREASE IN INTAKE	TOTAL EXCRETION	INCREASE IN EXCRETION	TOTAL INTAKE EXCRETED	INCREASE IN INTAKE EXCRETED
	μg	μg	μg	μg	%	%
I	600		113		19	
II	1017	417	150	37	15	9
III	1234	217	263	113	21	52
IV	7171	5937	4344	4081	61	69
V	1206		325		27	
V ¹	1230		289		23	

¹ Average if subject 2 is not included.

There was little variation in the average percentage of the total intake excreted, except during the period of supplementation. The figures on the percentage of total intake excreted probably are not so significant, however, as those on the percentage of the added riboflavin which was excreted in each period. When the riboflavin intake was increased from an average of 0.29 to 0.49 mg/1000 Cal. in period II (a total increase of about 400 μg per day) nearly all of the added riboflavin was "retained" by the body, only 9% of the added

amount being excreted. When 200 μ g per day were added in period III to make an intake of 0.66 mg/1000 Cal., more than 50% of the added amount was excreted. It seems logical to assume that when a relatively small increase in riboflavin intake results in an excretion of more than 50% of that increase, the added amount was not necessary.

The fact that both the daily urinary excretion and the test dose returns were increased when the average intake was 0.66 mg/1000 Cal. in period III, together with the fact that there was no apparent benefit from a 2-week period of vitamin supplementation, would indicate that this level of riboflavin intake probably is more than enough to take care of bodily needs for the vitamin. The observation that despite the low daily excretion of riboflavin, the fasting 1-hour excretion and the return of a test dose rose on an intake of 0.49 mg/1000 Cal. suggests that this level of intake supplies at least the minimal "requirements." This seems even more evident when it is considered that this level of intake followed a long period of low intake during which there was evidence that the tissue concentration of riboflavin had been decreased. This amount of riboflavin is the same as that found by Williams and his co-workers ('43) to supply the minimal requirements of riboflavin for women. This evidence, together with the data presented herewith which indicate that an intake of 0.66 mg/1000 Cal. supplied more of the vitamin than was necessary, strongly suggests that the riboflavin "needs" of healthy, active young women can be met by an intake of about 0.50 mg/1000 Cal., or a total intake of approximately 1 mg.

SUMMARY

The riboflavin excretion of young women on a diet in which the amount of the vitamin was progressively increased was followed during an 8-month period.

From an initial intake during periods I-a and I-b of 0.29 and 0.28 mg/1000 Cal., the riboflavin level was increased by

the addition of milk and by other dietary changes to 0.49 and 0.66 mg/1000 Cal. during periods II and III. In period IV, which lasted 2 weeks, the total daily intake was 7.1 mg. During period V, 3 weeks long, the intake averaged 0.63 mg/1000 Cal.

Physical examinations at the beginning of the experiment and following periods I-a and I-b revealed no signs of riboflavin deficiency.

Daily urinary excretions "levelled off" within 10 days after each change in diet. Average daily excretions during the first four periods were 119, 107, 150, and 263 μ g, respectively. Excretions rose sharply during supplementation, but after 2 weeks on a lower intake the average excretion was 325 μ g.

Levels of fecal excretion of riboflavin differed considerably among individuals, but the amount excreted by each person remained relatively constant despite dietary variations.

Test dose returns at the end of periods I-a and I-b averaged 2.8 and 4.5%. With increased intake in periods II and III, the average returns were raised to 11.8% and 14.3%, respectively. Test dose returns were not increased by supplementation, the average at the end of period V being 15.6%.

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